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Evaluation of biofilm formation on novel copper-catalyzed azide-alkyne cycloaddition (CuAAC)-based resins for dental restoratives

Sheryl Zajdowicz[a,*], *Han Byul Song*[b], *Austin Baranek*[b], *Christopher N. Bowman*[b]

[a] *Department of Biology, Metropolitan State University of Denver, PO Box 173362, Campus Box #53, Denver, CO, 80217, United States*

[b] *Department of Chemical and Biological Engineering, University of Colorado Boulder, 596 UCB, Boulder, CO, United States*

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ABSTRACT

Objective. For the past several decades, the resins used in dental restorations have been plagued with numerous problems, including their implication in biofilm formation and secondary caries. The need for alternative resins is critical, and evaluation of biofilm formation on these resins is essential. The aim of this study was to evaluate *in vitro* biofilm formation on the surface of novel copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC)-based resins and composites.

Methods. CuAAC-based resins/composites made from varying azide monomers and different copper concentrations were compared with BisGMA-TEGDMA resins/composites that served as the control. Biofilms were formed using a mono-species model containing a luciferase-expressing strain of *Streptococcus mutans*. Luciferase activity was measured and the number of viable bacteria was enumerated on biofilms associated with each resin and composite.

Results. A significant reduction ($p < 0.05$) in luciferase activity, and the number of viable bacteria recovered from biofilms on CuAAC-based resins and composites was observed in comparison to biofilms associated with the BisGMA-TEGDMA controls.

Significance. CuAAC-based resins do still allow for the formation of biofilms; however, the statistically significant reduction of growth that was associated with the CuAAC resin may enhance the longevity of restorations that incorporate CuAAC-based materials.

* *Corresponding author.*
E-mail addresses: swaltonz@msudenver.edu (S. Zajdowicz), han.song@colorado.edu (H.B. Song), austin.baranek@colorado.edu (A. Baranek), christopher.bowman@colorado.edu (C.N. Bowman).

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1. Introduction

The oral cavity is a complex environment where over 700 bacterial species have been detected in the oral common microbiota [1–4]. Within this diverse community of bacteria found in the mouth, *Streptococcus mutans*, due to its acidogenic nature and its ability to form biofilms on tooth surfaces, is one of the primary species associated with human dental caries and secondary caries formation [5,6]. Recent studies have also indicated that numerous other oral bacteria, most notably those that are acid producing, work together to form polymicrobial biofilms that ultimately initiate and further develop tooth decay [7–10]. In fact, *Lactobacillus acidophilus*, another commonly found oral acid-producing bacterium, has been found in high numbers in both superficial and deep dental caries [11,12], and its ability to form biofilms on tooth surfaces is enhanced by the presence of *S. mutans*, augmenting the ability to cause carious lesions [13,14].

More than 100 million dental restorations are performed each year and over 50% of these restorations utilize resin-based composites over amalgams [15–17]. Resin-based composites have many benefits over amalgams, including improved aesthetics, adhesive strength, and filling capability [16–18]. However, resin-based restoratives frequently have limited longevity due to restoration failure, commonly caused by degradation or fracture of the restoration directly or by failure due to secondary caries formation at the margins around the restoration [17,19–21]. Nearly all resin-based composite restoratives are methacrylate-based and consist of a co-monomer mixture comprised from components such as 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl] propane (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) [18,22] or related monomers. The functional integrity of these methacrylate restoratives relies on the polymerization of resin monomers, and their limited conversion leads not only deterioration of the restorative, but also to release of these monomers into the surrounding tissues [17,18,23,24]. Additionally, numerous studies have demonstrated sensitivity of these restorations in general, and these methacrylate monomers specifically, to hydrolysis by salivary and bacterial esterases found in the oral cavity, resulting in biodegradation by-products (BBPs) [25–31]. The biodegradation of these restorations increases bacterial leakage between resin-dentin interfaces, leading to further damage to the tooth [32]. Additionally, residual monomers released from resin restorations and resulting BBPs, such as methacrylic acid (MA), bishydroxypropoxyphenyl-propane (Bis-HPPP), and triethylene glycol (TEG), have been thoroughly investigated and implicated in adverse manifestations in the host. In particular, these effects include disruption of immune function [33–37], cytotoxicity [38–41], microbiota shifts [42], and accelerated formation of biofilms [43–46].

Due to the various pitfalls associated with the currently used methacrylate-based composites, the development of new, longer-lasting polymers for dental restoration is of utmost importance and could have a significant positive impact on global oral health. Since bacterial accumulation and biofilms have been implicated in the deterioration of the current BisGMA-TEGDMA based composites, novel resins that have structural stability and also limit bacterial prevalence are desirable to prolong the longevity of dental resins. Recently, novel resins have been developed that specifically have antibacterial properties to resist biofilm formation and incorporate antimicrobial monomers such as novel quaternary ammonium methacrylates [47–49], methacryloxylethylcetyl dimethyl ammonium chloride [50], and 12-methacryloyloxydodecylpyridinium bromide (MDPB) [51–54]. Additional strategies have also included the inclusion of alternative antimicrobial agents such as fluoride [49,55,56], silver nanoparticles [57–60], and chlorhexidine [61–63], to name a few. While providing additional benefits over the currently used resin-based composite systems, many of the approaches listed above continue to rely on the existing methacrylate system and, as such, have been plagued with similar challenges.

Recently, the development and analysis of novel visible light-initiated copper-catalyzed azide-alkyne cycloaddition (CuAAC)-based resins that possess superior mechanical properties, significantly reduced shrinkage stress and suitable polymerization kinetics as compared to BisGMA-based polymers [64–70]. However, the ability of these resins to promote or restrict bacterial growth has not been evaluated. Therefore, the aim of this study was to evaluate *in vitro* biofilm formation on the surface of novel copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC)-based resins.

2. Materials/methods

1,3-Bis(isocyanatomethyl)cyclohexane, 1,3-bis(2-isocyanatopropan-2-yl)benzene, 4,4-methylenebis(cyclohexyl isocyanate), 4,4′-methylenebis(phenyl isocyanate), dibutyltin dilaurate, tetrahydrofuran, 6-chloro-1-hexanol, sodium azide, 1,1,1-tris(hydroxymethyl)propane, propargyl bromide, propargyl alcohol, 3-(triethoxysilyl)propyl isocyanate, copper(II) chloride, N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA), camphorquinone (CQ), toluene, and acetonitrile were used as received from Sigma Aldrich. Propylamine, sodium hydroxide, dimethyl sulfoxide, dimethylformamide, methanol, and sodium sulfate were used as received from Fisher Scientific. The BisGMA/TEGDMA (70/30) comonomer mixture was used as donated from ESSTECH. Schott glass (mean particle size of 0.4 μm) with both untreated surface and surface treated with γ-methacryloxypropyltrimethoxysilane were used as received from ESSTECH. Syntheses of the azide monomers (AZ-1, AZ-2, AZ-3, AZ-4) and the alkyne monomer and alkyne silanization on glass microparticle (0.4 μm) were performed according to previously reported procedures [64,66]. All synthetic procedures along with NMR predictions and particle functionalization are provided in the Supporting Information. Structures for BisGMA and TEGDMA monomers used for methacrylate polymerization, the various azide monomers (AZ-1, AZ-2, AZ-3, AZ-4) and the alkyne monomer used for CuAAC polymerizations, as well as the copper catalyst $CuCl_2$[PMDETA] and photoinitiator CQ used in this study are shown in Fig. 1. Azides were synthesized according to the azide safety rules and handled with appropriate precaution when working with monomers, resins, and polymers in small quantities [71].